

WHAT IS CLAIMED IS:

1. A method for isolating a plant polynucleotide that comprises a T-DNA border-like sequence, comprising
  - (i) fragmenting a plant genome;
  - (ii) ligating a polynucleotide of known sequence to a plant DNA fragment to produce a ligated DNA;
  - (iii) producing a PCR product from the ligated DNA that comprises (a) a sequence that is homologous to a part of a T-DNA border sequence, (b) a DNA sequence from the plant genome, and (c) a sequence from the polynucleotide of known sequence, wherein the sequences of (a) and (b) are linked;
  - (iv) sequencing the PCR product;
  - (v) designing at least one PCR primer based on the DNA sequence from the plant genome;
  - (vi) using the PCR primer of (v) in an inverse PCR of plant genomic DNA to identify a sequence from the plant genomic DNA that is a T-DNA border-like sequence.
2. The method of claim 1, wherein the PCR product of step (iii) is produced by a primer pair, of which, one primer has a sequence comprising 5'- YGR CAG GAT ATA T-3 or 5'-CAG GAT ATA TNN NNN KGT AAA C-3'.
3. A method for producing a modified plant that does not contain a T-DNA comprising (1) transforming a plant cell with (i) an *Agrobacterium*-transformation vector that comprises a desired polynucleotide within a P-DNA, and (ii) an *Agrobacterium*-transformation

vector that comprises a selectable marker within a T-DNA; (2) obtaining from said transformed plant cell a transformed plant that comprises at least one copy of said P-DNA and at least one copy of said T-DNA in its genome; (3) self-fertilizing or cross-fertilizing the transformed plant to produce progeny plants that segregate for the T-DNA and P-DNA; and (4) screening the progeny plants to identify a modified plant that does not comprise said T-DNA, but does comprise said P-DNA.

4. A modified tuber, comprising a level of acrylamide that is at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% lower than the level of acrylamide normally associated with a wild-type tuber of the same species as the species of the modified tuber.

5. The modified tuber of claim 4, wherein the modified tuber is a mature tuber.

6. The modified tuber of claim 4, wherein the modified tuber is at least 12-weeks old.

7. The modified tuber of claim 4, wherein the tuber is selected from the group consisting of ahipa, apio, arracacha, arrowhead, arrowroot, baddo, bitter casava, Brazilian arrowroot, cassava, Chinese artichoke, Chinese water chestnut, coco, cocoyam, dasheen, eddo,

elephant's ear, girasole, goo, Japanese artichoke, Japanese potato, Jerusalem artichoke, jicama, lilly root, ling gaw, mandioca, manioc, Mexican potato, Mexican yam bean, old cocoyam, potato, saa got, sato-imo, seegoo, sunchoke, sunroot, sweet casava, sweet potatoes, tanier, tannia, tannier, tapioca root, topinambour, water lily root, yam bean, yam, and yautia.

8. The modified tuber of claim 7, wherein the potato is a Russet potato, a Round White potato, a Long White potato, a Round Red potato, a Yellow Flesh potato, or a Blue and Purple potato.

9. A modified tuber comprising a level of amylose that is at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% greater than the level of amylose of a wild-type tuber of the same species as said modified tuber.

10. A modified, mature tuber comprising a level of amylose that is about 1 times, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, or 10 times greater than the level of amylose of a wild-type tuber of the same species.

11. The modified tuber of claim 9, wherein the modified tuber is a mature tuber.

12. The modified tuber of claim 9, wherein the modified tuber is at least 12-weeks old.

13. A modified tuber comprising a level of cold-induced glucose that is at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% lower than the level of glucose in a wild-type tuber of the same species as said modified tuber.

14. The modified tuber of claim 13, wherein the level of glucose in the modified tuber is about 40% lower than the level of glucose in the wild-type tuber of the same species.

15. A modified, mature tuber comprising a 5-fold reduction in acrylamide levels compared to the level of acrylamide in a wild-type tuber of the same species.

16. The modified tuber of claim 13, wherein the modified tuber is a mature tuber.

17. The modified tuber of claim 13, wherein the modified tuber is at least 12-weeks old.

18. A modified tuber comprising a level of phosphate that is at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%,

89%, 88%, 87%, 86%, 85%, 84%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% lower than the level of phosphate in a wild-type tuber of the same species as said modified tuber.

19. The modified tuber of claim 18, wherein the modified tuber is a mature tuber.

20. The modified tuber of claim 18, wherein the modified tuber is at least 12-weeks old.

21. A modified tuber comprising a level of polyphenol oxidase activity that is at least about 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% lower than the level of polyphenol oxidase activity associated with a wild-type tuber of the same species as the species of the modified tuber.

22. A modified tuber comprising at least one cell that overexpresses an inactive polyphenol oxidase gene, wherein the level of polyphenol oxidase activity in the modified tuber is reduced by 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%, 66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% in comparison to the level of polyphenol oxidase activity in a wild-type tuber of the same species as the modified tuber.

23. The modified tuber of claim 22, wherein the modified tuber is a mature tuber.

24. The modified tuber of claim 22, wherein the modified tuber is at least 12-weeks old.

25. The modified tuber of claim 22, wherein the level of polyphenol oxidase activity in the modified tuber is reduced by 50%-90%.

26. The modified tuber of claim 23, wherein the level of polyphenol oxidase activity in the modified tuber is reduced by 50%-90%.

27. The modified tuber of claim 23, wherein the level of polyphenol oxidase activity in the modified tuber is reduced by 99%, 98%, 97%, 96%, 95%, 94%, 93%, 92%, 91%, 90%, 89%, 88%, 87%, 86%, 85%, 84%, 84%, 83%, 82%, 81%, 80%, 79%, 78%, 77%, 76%, 75%, 74%, 73%, 72%, 71%, 70%, 69%, 68%, 67%,

66%, 65%, 64%, 63%, 62%, 61%, 60%, 59%, 58%, 57%, 56%, 55%, 54%, 53%, 52%, 51%, 50%, 49%, 48%, 47%, 46%, 45%, 44%, 43%, 42%, 41%, 40%, 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%, 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, or 1% in comparison to the level of polyphenol oxidase activity in a wild-type tuber of the same species as the modified tuber.

28. A method for producing a transgenic plant that does not contain a T-DNA, comprising

co-transforming a plant tissue with a P-DNA vector and a T-DNA vector;

growing plantlets from the transformed plant tissue by incubating the plant tissue on media that contains a substance that destroys cells which do not contain the P-DNA vector or the T-DNA vector;

selecting a plantlet that contains either or both of the P-DNA or the T-DNA;

growing the plantlet to reproductive maturity and cross-fertilizing the reproductively mature plant with an untransformed plant; and

segregating progeny plants whose cells contain only a P-DNA from progeny plants that contain a T-DNA.

29. The method of claim 28, wherein the P-DNA vector is substantially similar to pSIM340.

30. The method of claim 28, wherein the T-DNA vector is substantially similar to pSIM363.

31. The method of claim 28, wherein a substance in the media is timentine.

32. The method of claim 28, wherein a substance in the media is kanamycin.

33. A method for producing a transgenic plant that does not contain a T-DNA, comprising

co-transforming a plant tissue with a P-DNA vector and a T-DNA vector;

growing plantlets from the transformed plant tissue by incubating the plant tissue on media that contains a substance that destroys cells which do not contain the P-DNA vector or the T-DNA vector;

selecting a plantlet that contains either or both of the P-DNA or the T-DNA;

growing the plantlet to reproductive maturity and then self-fertilizing the reproductively mature plant; and

segregating progeny plants whose cells contain only a P-DNA from progeny plants that contain a T-DNA.

34. The method of claim 33, wherein the P-DNA vector is substantially similar to pSIM340.

35. The method of claim 33, wherein the T-DNA vector is substantially similar to pSIM363.

36. The method of claim 33, wherein a substance in the media is timentine.



37. The method of claim 33, wherein a substance in the media is kanamycin.

38. The method of claim 28 or 33, wherein the T-DNA comprises a selectable marker gene.

39. A method for producing a transgenic plant that does not contain a T-DNA, comprising

co-transforming a plant tissue with a first P-DNA vector and a second P-DNA vector, wherein the first P-DNA vector comprises a polynucleotide of interest, and wherein the second P-DNA vector comprises a selectable marker gene;

growing plantlets from the transformed plant tissue by incubating the plant tissue on media that contains a substance that destroys cells which do not contain either of the P-DNA vectors;

selecting a plantlet that contains either or both of the P-DNA vectors;

growing the plantlet to reproductive maturity and either cross-fertilizing the reproductively mature plant with an untransformed plant or self-fertilizing the reproductively mature plant; and

segregating progeny plants, whose cells contain only the P-DNA from the first P-DNA vector, from progeny plants that contain in their genomes the P-DNA from the second P-DNA vector.

40. A nucleic acid comprising the sequence depicted in SEQ ID NO. 96, and is capable of transferring one polynucleotide into another polynucleotide.

41. A nucleic acid comprising a sequence that has 90% sequence identity to the sequence depicted in SEQ ID NO. 96.

42. A nucleic acid comprising the sequence depicted in SEQ ID NO. 97, and is capable of transferring one polynucleotide into another polynucleotide.

43. A nucleic acid comprising a sequence that has 90% sequence identity to the sequence depicted in SEQ ID NO. 97.